Docket No.: 09600-00028-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Klaus Henning

Application No.: 10/524,424 Confirmation No.: 6261

Filed: July 22, 2005 Art Unit: 1623

For: HIGHLY BRANCHED, UNSUBSTITUTED OR LOW-SUBSTITUTED STARCH PRODUCTS, DIALYSIS SOLUTION AND PLASMA EXPANDER CONTAINING THE SAME, AND THE USE THEREOF Examiner: E. Olsen

RULE 1.132 DECLARATION OF DR. KLAUS HENNING

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Dr. Klaus Henning, am a citizen of Germany and reside at Landrat-Beckmann-Str. 21, D-61250 Usingen, Germany, hereby declare and say as follows:

1. I am a fully trained chemist, having studied Chemistry at the University of Würzburg, in Würzburg, Germany. In addition, I am well acquainted with technical English.

2. I have been working for Fresenius Kabi Deutschland GmbH since 1987. I am a technical expert in the field of Carbohydrate Chemistry, with a particular expertise in the production of starch products for the use as volume expanders. I have worked and performed research in the field of Carbohydrate Chemistry since 1987.

3. In the field of Carbohydrate Chemistry, I am an inventor on four U.S. patents and patent applications and an author of one publication.

4. In view of my qualifications, as outlined above, I consider myself to be an expert in and to be skilled in the art of Carbohydrate Chemistry.

5. A toxicokinetic study was performed under my supervision by personnel of the clinical research department of Fresenius. I have evaluated the results of this study. This study was performed to compare the storage effects of the following products:

   a. Inventive Example 1 (IE 1) – a hyperbranched hydroxyethyl starch (HB-HES) product having (1) an average molecular weight (M_W) of 106,933 Dalton, (2) a degree of branching in the range of from 8 to 10 mol %, and (3) a degree of substitution (MS) of 0.06;

   b. Inventive Example 2 (IE 2) – an HB-HES product having (1) an average M_W of 123,233 Dalton, (2) a degree of branching in the range of from 8 to 10 mol %, and (3) an MS of 0.1;

   c. Inventive Example 3 (IE 3) – an unsubstituted hyperbranched (HBS) product having (1) an average M_W of 127,667 Dalton, (2) a degree of branching of 8-10%, and (3) an MS of 0;

   d. Comparative Example 1 (CE 1) – an HES product having (1) an average M_W of 181,900 Dalton, (2) a degree of branching of approximately ~5%, and (3) an MS of 0.041;

   e. Comparative Example 2 (CE 2) – an unsubstituted starch product having (1) an average M_W of 204,933 Dalton, (2) a degree of branching of approximately ~5%, and (3) an MS of 0;

   f. Comparative Example 3 (CE 3) - an already existing product HES 130/0.4 named Voluven 10 % which is specified in the range of M_w 130,000 Dalton, MS = 0.40; this product has also a natural branching degree of about 5% .

6. The study was performed according to the following procedure. Ten percent saline solutions of each of the above products were prepared and infused into rats over the course of 3 hours in an amount of 90mL/kg body weight. Post infusion plasma levels of

the products (i.e., concentration of each product in the body) were determined at 0 hour and at 8 hours after termination of infusion. The study was carried out under GLP conditions to guarantee correctness of the plasma level values.

7. The results of this study are as follows:

Example	IE 1	IE 2	IE 3	CE 1	CE 2	CE 3
Plasma concentration at 0 hour [mg starch product / ml plasma]	7.42	8.03	1.81	0.91	1.40	23.50
Plasma concentration at 8 hours [mg starch product / ml plasma]	0.03	0.77	0.19	0.83	0.86	1.96

The product plasma concentration is represented in units of mg of starch product / mL of plasma. Each value is an average of three experiments.

8. The data above clearly demonstrate that the claimed products possess unexpectedly superior storage effects. Both IE 1 and IE 2, which each have a degree of branching in the range of from 8 to 10 mol %, exhibit a substantially smaller storage effect (0.03 and 0.77, respectively) 8 hours after termination of infusion compared to CE 1 (0.83), which has a conventional degree of branching of approximately ~5%. This is unexpected since an increased storage effect would have been expected for the more highly branched starches IE 1 and IE 2. Enzymatic degradation of a starch occurs when amylase attacks the linear α-1,4-bond between its glucose monomers. Increased branching of the starch would be expected to interfere with the attachment of amylase at the relevant structure sub-unit due to increased steric hindrance, resulting in a much slower rate of degradation. The slower the rate of enzymatic degradation of the starch, the longer the expected tissue storage effect of the starch. In view of this, the skilled artisan would have expected IE 1 and IE 2 to exhibit a longer storage effect compared to CE 1. Surprisingly, I observed

that the storage effects of IE 1 and IE 2 are substantially reduced compared to CE 1. Furthermore, it is well known in the art that tissue storage of a substituted starch increases as its degree of substitution (MS) is increased, since the concomitant increase in steric hindrance interferes with enzymatic degradation of the substituted starch. Conversely, lowering the MS reduces this interference. Increasing the degree of branching of a substituted starch would be expected to mitigate the reduced interference with enzymatic degradation that would result from deceasing its MS. However, instead of mitigating the reduced tissue storage expected from decreasing the MS, I surprisingly observed that increasing the branching beyond a conventional degree dramatically reduces tissue storage. Compare IE 2, which has a branching degree of 8 to 10 mol % and an MS of 0.1, with CE 1, which has a branching degree of approximately 5% and an MS of 0.041. Even though the MS of IE 2 is more than twice that of CE 1 and the branching degree of IE 2 is 60 to 100% greater than that of CE 1, the plasma concentration of IE 2 is more than 7% lower than that of CE 1 after 8 hours.

9. This unexpectedly superior storage effect is attributable to the higher than conventional degree of branching (i.e., in the range of from 8 to 20 mol %), since it is independent of the degree of substitution. This is demonstrated by comparing IE 3 to CE 2. Both IE 3 and CE 2 have the same MS (0), but IE 3 has a degree of branching in the range of from 8 to 10 mol % while CE 2 has a conventional degree of branching of approximately ~5%. However, the product plasma concentration at 8 hours after termination of infusion was 0.86 for CE 2, but only 0.19 for IE 3.

10. In view of the above data, it is clear that the three embodiments of the claimed products (IE 1, IE 2 and IE 3) exhibit a substantially smaller tissue storage effect after 8 hours compared to the conventional product CE 3. This demonstrates the surprising effect of the high degree of branching on the tissue storage independent of the degree of substitution. As such, the skilled artisan would not have expected the same effect from the starches disclosed in U.S. Patent Nos. 6,284,140 and 5,218,108.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Klaus Henning

02.10.2008

Date